CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

SEMI-MICRODROPLET ASSAY

FOR CELL ADHESION MOLECULES

A thesis submitted in partial satisfaction of the
requirements for the degree of Master of Science in

Biology

by

Lawrence Shinzo Tava

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ABSTRACT

SEMI-MICRODROPLET ASSAY

FOR CELL ADHESION MOLECULES

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A new cell-to-cell adhesion assay has been devised. Using dissociated embryos of the sea urchin Strongylocentrotus purpuratus, this procedure involves rotating a 0.100 ml suspension of single cells (500,000/ml) with 0.100 ml of the solution to be tested in the bulb portion of a transfer pipet with the tip removed. After 1 hour of rotation at 60 rpm at 15 C, the contents of each bulb were transferred into individual wells of a 96-well flat-bottom plate. After the plate was incubated for 1 hour at 15 C, black and white photographs were taken with a 35 mm camera attached to an inverted photomicroscope. Examining a proof sheet i.e., of contact prints, or the negatives directly allowed rapid evaluation of suspected cell adhesion promoting factors. A ranking system was used to evaluate all samples. The assay was tested by

examining the effect of specific solutions i.e., calcium-magnesium free-sea water with or without bovine serum albumin, millipore-filtered sea water, or supernatant containing cell adhesion molecules colored with or without phenol red, on the aggregation of single cells obtained from dissociated 23-hour S. purpuratus embryos. Samples were scored as negative when single cells with no clumping were observed while clumping indicated a positive response; larger clumps were scored as increasingly positive. This serological assay differs from other adhesion assays in that it utilizes the rotation of small volumes of cell suspension, employs a scoring method based on the largest clumps observed, and requires only minimal laboratory experience and completely disposable supplies.

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The thesis of Lawrence Shinzo Tawa is approved:

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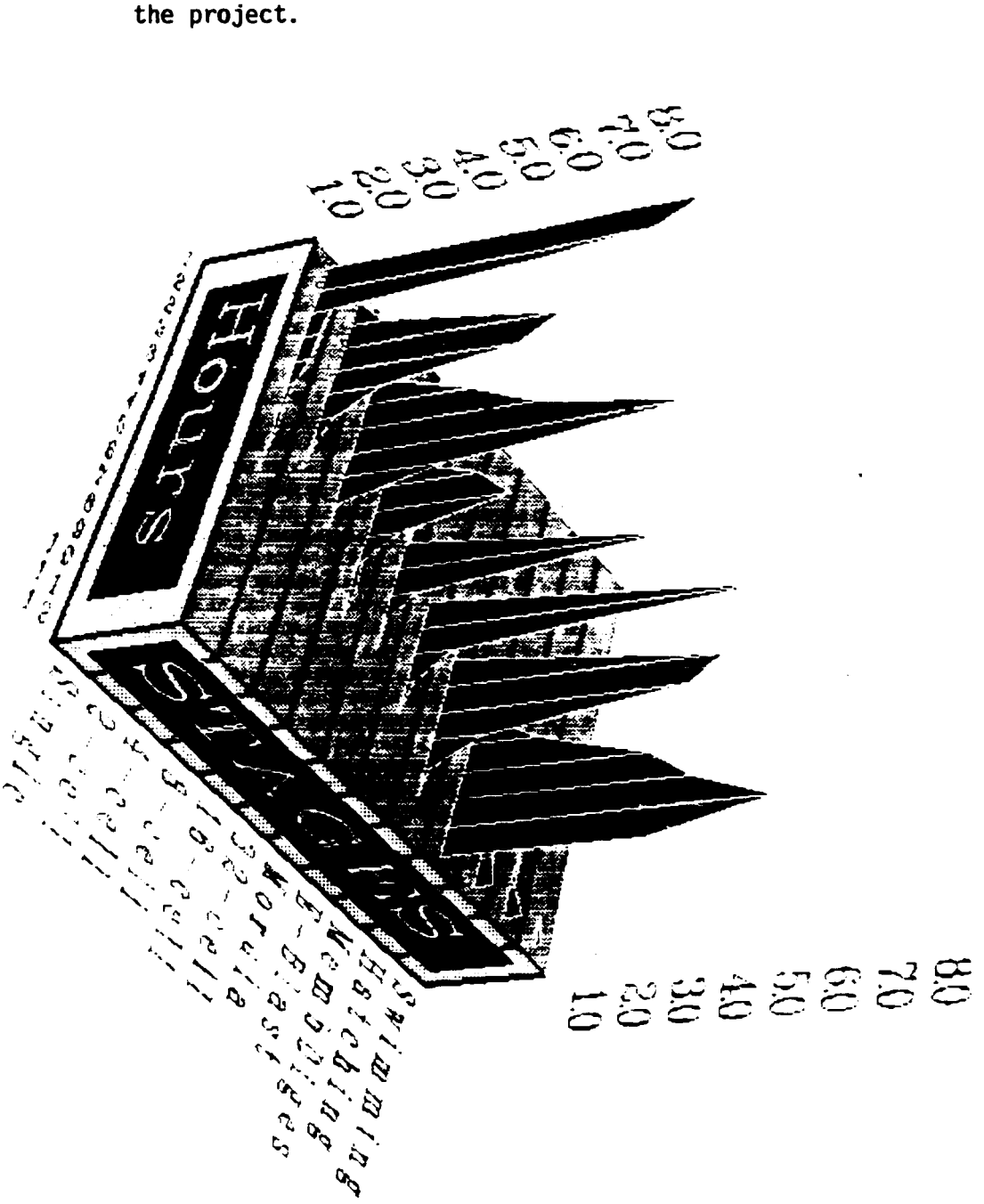
The following pages represent our first attempts at a computer graphics analysis of sea urchin embryo development. The graphs represent our preliminary data on the time course of development. A complete explanation of the graphics program and details of each graph will be presented by John Slack in a seminar at NASA-AMES and/or in a paper on the work that will be written in collaboration with NASA-AMES at the completion of this phase of the project.



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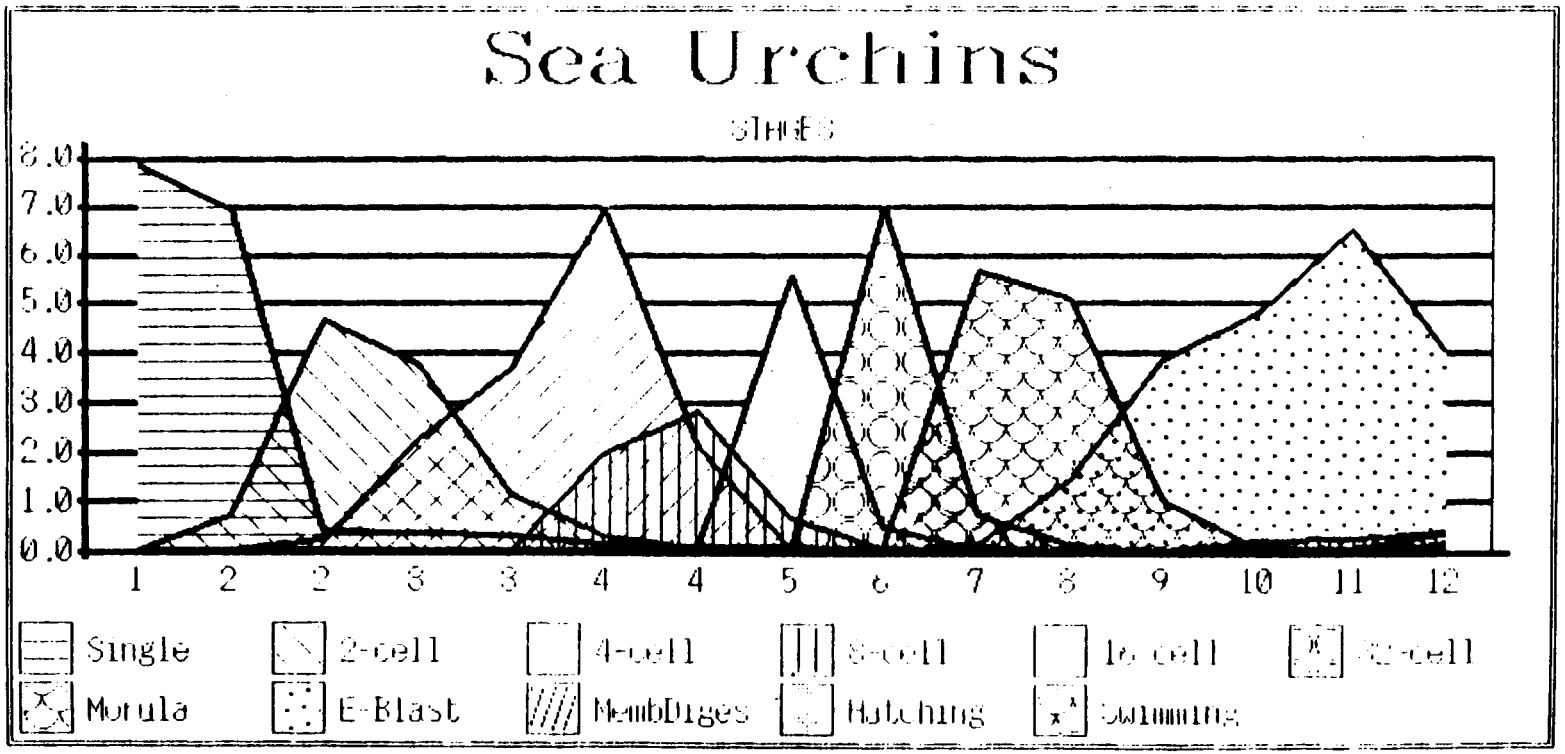

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